REMARKS

Claims 1-5, 7, 9-12, 25-28, 48 and 51-54 are pending in the subject application. Applicants have no amended or canceled any claims.

1. Reference Cited By Applicants

The following is a list of references, attached hereto as **Exhibits A-E**, which are referenced by applicants throughout this Communication.

Exhibit A :	Gronthos S., et al., Journal of Cellular Physiology 189 :54-63 (2001)
Exhibit B :	Katz et al., 2005, Stem cells, 23, 412-423 (2005)
Exhibit C :	Strem BM, et al., 2005, Keio J Med, 54(3), 132-141 (2005)
Exhibit D :	Wagner et al., 2005 Experimental Hematology, 33, 1402-1416 (2005)
Exhibit E :	Bernardo M.E. et al., Cancer Research 2007, 67; (19), 9142-9149

A Supplemental Information Disclosure statement, in which the Examiner is requested to make the above references of record, begins on page 12 of this Communication.

2. Claim Rejections 35 USC §103

The Examiner has maintained the rejection of claims 1-5, 7, 9-12, 25-28, 48, 51-54 as filed on July 7, 2009 under 35 U.S.C. 103(a) as allegedly unpatentable over Katz et al., previously cited, Akanbi et al., previously cited, Hedrick et al., previously cited, Haynesworth et al., previously cited, Tremain et al., newly cited, Djian et al., newly cited, and Young et al., newly cited.

As a preliminary remark, it is noted that seven documents are used to formulate the objection under 35 U.S.C. 103(a). The skilled person would not combine the teaching of such a high number of documents in an obvious manner. Applicants submit that, in addition to the

comments below, the high number of references is an indication that the objection is not well founded.

2.1 Newly cited references

2.1.1 Tremain et al.

The Examiner states, with regard to the steps c) and d) of claim 25, that stem cell lines originate from a single cell and that Tremain et al. teaches that single cell derived colonies can be isolated by using cloning cylinders and that the single cells can be tested for colony forming efficiency which is a predictor for life span and differentiation potential of the cell.

In response, it is noted that Tremain et al. is relative to isolation of stem cells from bone marrow, more specifically from the iliac crest of human donors (see page 409, 2nd column under Isolation and Culture of Human Stem Cells). Tremain et al. does not provide any teaching relative to the isolation of stem cells from human adipose tissue, let alone to the obtaining of single cell derived colonies from human adipose tissue. Adipose tissue has a structure which is different from that of bone marrow, in particular the stem cells are far more diluted than in bone marrow (see for example enclosed **Exhibit E** (Bernardo et al), which states at page 9148, column 2, lines 6 to 9: "indeed, in comparison with BM, which is very rich in stem cells, fat tissue contains mainly differentiated cells, and it generates MSC's that are immunophenotypically slightly different from those derived from BM."

Thus the non obvious step performed in the method according to the present invention is the obtaining of a cell population which originates from adipose tissue and in which the concentration of stem cells is high enough for stem cells clones to be obtained. Tremain et al. does not address this problem because Tremain et al. is not relative to stem cells from adipose tissue. Thus applying the teaching

of Tremain et al. does not allow to obtain a cell population which originates from adipose tissue and in which the concentration of stem cells is high enough for stem cells clones to be obtained.

2.1.2 Djian et al.

The Examiner states that Djian et al. teaches that a period of 12 hours is sufficiently long to allow adherence of cells but is too brief for appreciable replication. The Examiner derives from this teaching that the artisan would plate the cells for 12 hours because allowing cells to adhere for 12 hours maximizes the number of cells that adhere to the plate and minimizes any confusion that "two cells sitting next together are two different cells that plated next to each other or is the result of cell division".

The relevance of the hereabove underlined part of the Examiner's reasoning to step (c) of claim 25 is not entirely clear to the Applicant. However it is noted in any event that the passage of Djian et al. cited by the Examiner (page 1201, first paragraph of second column) is relative to the cloning of adipocyte precursors. As previously and repeatedly exposed by the Applicant, adipocyte precursors are **not** stem cells. Their features are very different. In particular, their differentiation and self-renewal potential is much more limited. Djian et al. provides no teaching as to the cloning of stem cells. The skilled person would thus not consider that a method of cloning adipocytes can be applied to clone stem cells. Rather, the skilled person would consider that applying the method described in Djian et al. would lead him to obtain adipocyte precursors. He would thus disregard the teaching of Djian et al., because his objective is to obtain stem cells, not adipocyte precursor cells.

2.1.3. Young et al.

The Examiner considers that Young et al. is relevant for step e) of claim 25 which recites that the CA cells are cultured for 50-80 population doublings and are diluted by a maximum of two to three at each transfer. The Examiner states that the abstract of Young et al. teaches that primitive quiescent cells are retained in bulk expansion cultures and that the cell production capacity of the expanded cell product can largely be attributed to cells exhibiting quiescent to cells exhibiting quiescent behaviour during culture. The Examiner further states that quiescent cells were shown to proliferate more and produced progeny that included multilineage colony forming cells, that these cells were thus demonstrated to be stem cells, and that given these teachings, an artisan would have performed bulk culture such as taught by Young et al., in order to identify stem cells.

It is not entirely clear to the Applicant why the Examiner considers the abstract of Young et al. (or any other part of this reference) to be relevant to step e) of claim 25. Indeed, step e) of claim 25 does not recite the performing of bulk culture. Further, Young et al. neither discloses nor suggests that a cell population comprising stem cells, let alone a cell population derived from adipose tissue, be cultured for 50-80 population doublings and be diluted by a maximum of two to three at each transfer. Young et al. discloses that proliferation of quiescent cells can be performed in a specific single cell proliferation medium, and that the average production per input cell is 1780 cells, that is approximately 11 population doublings (2^{11} = 2048) (see page 550). Further, this proliferation step is performed after a quiescent state is reached, not before as recited in step e) of claim 25. Finally, it is concluded by Young et al. that "It remains to be shown whether enough quiescent stem cells can be maintained in bulk culture to provide long-term engraftment" (see page 555).

Thus, the method disclosed by Young et al. does not comprise the culture of a population of cells, let alone cells obtained from adipose tissue, for 50-80 population doublings as recited in step e)

of claim 25. Its teaching is thus not relevant for the assessment of the patentability of step e) of claim 25. Further, Young et al. does not disclose a means to obtain a high number of stem stems. The skilled person would thus disregard the teaching of Young et al., because his objective is to obtain a high number of stem cells.

2.2 Examiner's comments on Applicants arguments filed on July 7, 2009.

Applicant maintains the arguments filed on July 7, 2009. The Examiner stated that these arguments are not persuasive. In response, it is submitted that at least part of the assertions made by the Examiner are either incorrect or not relevant for the assessment of non-obviousness.

2.2.1 The prior art does not provide guidance to obtain the claimed cell

The Examiner first stated that the art provides guidance for an artisan to make single cell clones such that stem cells can be identified. The Examiner in particular stated that Tremain et al. teach that it is routine for an artisan to select single cells and test the for colony-forming efficiency and that an artisan would have performed the bulk culture method taught by Young et al., in order to confirm that the cells identified as stem cells in Tremain's assay are in fact stem cells.

In response, as stated above, Tremain et al. is relative to isolation of stem cells from bone marrow, not from adipose tissue. Tremain et al. does not provide any teaching relative to the isolation of stem cells from human adipose tissue, let alone to the obtaining of single cell derived colonies from human adipose tissue. Further, as also noted above, the method disclose by Young et al. does not comprise the culture of a population of cells, let alone cells obtained from adipose tissue, for 50-80 population doublings as recited in step e) of claim 25. It is thus respectfully submitted that the teaching of

Tremain et al. and Young et al. would not lead the skilled person to perform steps a) to g) as recited in claim 25.

2.2.2 The claimed cells are HLA class I negative

The Examiner also stated that the USPTO can require an applicant to prove that the prior art products do not necessarily or inherently possess the characteristic of the claimed product.

Without conceding the correctness of the Examiner's statement, Applicant attaches herewith references which show that so-called "adipose-derived stem cells" capable of differentiating into several mesodermal cell lines and obtained by methods disclosed in the prior art are HLA class I positive :

- Gronthos et al., 2001 (**Exhibit A**),
- Katz et al., 2005 (**Exhibit B**),
- Strem et al., 2005 (**Exhibit C**), and
- Wagner et al., 2005 (**Exhibit D**),

(see in particular Exhibit A, page 57, right hand column, first full paragraph; Exhibit B, page 416, right col. and table 3; Exhibit B, page 133, right hand column, Exhibit D, page 1405, right hand column, last paragraph).

Thus, it can be concluded that cells presented in the literature as "adipose-derived stem cells" are **HLA-class I positive**. **Those of the invention, on the contrary, are HLA-class I negative.**

Katz et al. (WO 00/53795) provide no information relative to the HLA Class I phenotype of the cells they disclose. However, it is noted that the HLA-class I positive "adipose-derived multipotent stem cells" disclosed in Exhibits A, B, C and D :

- are obtained, in the same way as the cells disclosed in Katz et al. (WO 00/53795), from liposuction tissue obtained from patients undergoing elective surgery, i.e. from adults (see in particular Exhibit A, paragraph bridging pages 55 and 56; Exhibit B, page 414, left hand column, first paragraph; Exhibit C, page 132, right hand column; Exhibit D, page 1403, right hand column, fourth paragraph);
- are isolated, in the same way as the cells disclosed in Katz et al. (WO 00/53795), through a process comprising liposuction, tissue washing, enzymatic digestion, centrifugation and erythrocytes lysis (see in particular Exhibit A, paragraph bridging pages 55 and 56; Exhibit B, page 414, left hand column, second paragraph; Exhibit C, page 132, right hand column; Exhibit D, page 1403, right hand column, fourth paragraph), but not comprising a selection step based on the adherence capacity after 12 hours, an enrichment step until quiescence is reached and the induction of proliferation after quiescence;

In contrast, as stated above, the cells of the invention are derived from human adipose tissue of a child under 10 years of age and were isolated by a method comprising a selection step based on the adherence capacity at 12 hours or less, an enrichment step until quiescence is reached and the induction of proliferation after quiescence, thus achieving concentration of the number of true stem cells. It is on attaining quiescence that the cell population becomes homogeneous and presents an HLA Class I negative phenotype (see present application, page 14, lines 18 to 21).

Importantly, the origin of the cells of Katz et al. (WO 00/53795) and the method of obtaining them are much more similar to that of Exhibits A, B, C and D than to that of the cells of the invention. Therefore, as the cells disclosed in Exhibits A, B, C and D are HLA Class-I positive, it cannot reasonably be concluded, that the cells of Katz et

al. (WO 00/53795) are inherently HLA-class I negative and cannot be distinguished from the cells of the invention.

It is considered the above cited references provide sufficient evidence that the cells obtained according to the method of claim 25 are different from the prior art cell, in particular with respect to their HLA class I phenotype.

2.2.3 Surprising effect

The Examiner further stated that the identification of a new characteristic of a known product does not render the instant invention novel. The Examiner also states that Young et al. teach that bulk culture identifies quiescent stem cells.

In response, it is noted as stated above in section 2.2.2, that the known "adipose derived stem cells" have a phenotype which is MHC (or HLA) class I positive. The claimed cells are different from the prior art cells in that they are MHC class I negative. The MHC class I phenotype is thus not "a new characteristic of a known product" but a characteristic of a new product.

As detailed in Applicant's previous response, the inventors have discovered that the MHC-I phenotype of the CA cell population obtained after performing steps a) to d) is positive and that it becomes negative during the course of step e). This change of phenotype is neither taught nor suggested in the prior art. In particular, as indicated above, Young et al. does not tech the performing 50-80 population doublings. The skilled person would thus not have performed this step in an obvious manner.

The effect of the number of population doublings on the MHC-I phenotype of the CA population was thus totally unexpected.

2.2.4 Akanbi et al.

The Examiner further maintained that the combined teaching of Katz et al. for teaching that stem cells can be obtained from human adipose tissue and Akanbi et al. for teaching that adipose precursor cells from young animals replicate faster and/or contain more clones capable of full differentiation into adipocytes than cells from older animals, would lead the skilled artisan to perform step a). The Examiner also does not agree with the Applicant's argument that Akanbi et al. teach away from using adipose tissue from a younger subject and stated that he was not relying on Akanbi et al. for teaching adipose precursor cells, but rather for teaching that there are different characteristics in adipose precursor cells obtained from newborn animals versus that of adult animals and that cells from newborns have a better potential for producing a large quantity of differentiated cells. The Examiner also stated that Akanbi et al.'s teaching does not teach away from the claimed invention because ultimately, an artisan would want stem cells whose descendants produce large amounts of differentiated cells.

It is respectfully submitted the Examiner appears to misunderstand the objective of the present invention. The objective of the present invention is certainly **not** to produce a large quantity of differentiated cells. The objective of the present invention is to produce a large quantity of stem cells, that is a large quantity of **un**differentiated cells. Further, the objective of the invention is not either to produce a large quantity of adipose precursor cells because these cells do **not** have the potential to differentiate in cell types from lineages other than the adipose lineage. The objective of the present invention is to produce a large quantity of stem cells which, because they are stem cells, can differentiate in cells types from an important number of different lineages. The precursor cells disclosed in Akanbi et al. only have the potential to produce large quantities of adipocytes but not cells from other lineages. Applicant thus

maintains that Akanbi et al. teach away from using adipose tissue from a younger subject.

3. CONCLUSION

In conclusion, it is maintained that the claimed cells are not obvious over the prior art for the reasons detailed in Applicant's response dated July 7, 2009 and further detailed herein.